PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	John G. Babish, *et al.*
Application No.:	10/789,814
Filing Date:	February 27, 2004
Docket Number:	068911-0075
Title:	SYNERGISTIC ANTI-INFLAMMATORY PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE
Examiner:	Shobha Kantamneni
Art Unit:	1617

CERTIFICATE OF TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to MAIL STOP AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date indicated below.

Date: 08/07/06

Angelo J. Mignanelli
Erin M. Olson

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Sir:

DECLARATION PURSUANT TO 37 C.F.R. § 1.131

I, John G. Babish declare as follows:

1) I am Dr. John G. Babish, Executive Vice President of Metaproteomics, LLC. I have held this position since August 2002.

2) I have Doctorate and Masters degrees, respectively, in Biochemistry and Chemistry from Cornell University, as well as a Bachelor degree in Biochemistry from The Pennsylvania State University. A copy of my Curriculum Vitae is attached as Exhibit A.

3) I am also an inventor named in several domestic and foreign patent applications including U.S. Application Nos. 10/141,085; 10/789,814; 10/789,817; 10/988,393; 10/480,145; 10/484,123; 10/881,404; 10/774,048; 10/464,834; 10/234,002 and 09/952,632 and issued foreign and domestic patents, including U.S. Patent Nos. 6,140,063; 5,506,420; 6,629,835; 6,733,793 and 6,908,630.

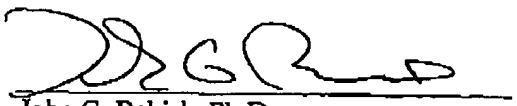
4) On the basis of 30 years of training and experience, I am an expert in the art of molecular biology, more specifically, that aspect of molecular biology involving signal transduction. I was a faculty member at the College of Veterinary Medicine, Cornell University for 17 years. As Professor of Pharmacology and Toxicology, my research program involved the elucidation of mechanisms by which xenobiotics affect signaling pathways in normal and transformed cells. Using the tools of molecular biology such as monoclonal antibodies, northern and western blotting and enzyme-linked immunoassays, my research program developed cell-based assays for the identification of small molecules directed at inhibiting selected cellular functions. Findings from these studies were used to identify potential anti-viral and anti-neoplastic pharmacophores from natural products. My research has also identified both positive and negative drug-drug and drug-nutrient interactions.

5) I understand that in the course of the February 7, 2006 Office Action during prosecution in the above-captioned application, Examiner Shobha Kantamneni rejected claims 1 – 7 under 35 U.S.C.§ 103(a), as allegedly being unpatentable over Kuhrts (US 2004/0137096, PTO-892) for reasons of obviousness. I respectfully submit that the instant invention was conceived prior to the January 9, 2003 filing date of the cited Kuhrts application and diligently researched until the February 27, 2004.

6) The basis to assert that the instant invention was invented prior to the Kuhrts reference cited is supported by copies of laboratory notebook pages dated from June 2002 through December 2003 showing research on the synergistic, anti-inflammatory effects of reduced isoalpha acids (RIAA) and isoalpha acids (IAA). Such support documentation is appended herewith as Exhibit B.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5-14-06



John G. Babish, Ph.D.
Executive Vice President
Metagenics Research Center - Suite 100
9770 44th Ave. NW
Gig Harbor, WA 98332

[illegible]

Exhibit A

BIOGRAPHICAL SKETCH AND BIBLIOGRAPHY

John G. Babish — Chariperson, BIOnexus, Ltd.
Executive Vice President, Metaproteomics Inc.

Education

Institution and Location of Study	Degree	Date Conferred	Field
The Pennsylvania State University, State College, PA	B.S	1968	Biochemistry
Cornell University, Ithaca, NY	M.S	1974	Chemistry
Cornell University, Ithaca, NY	Ph.D.	1976	Biochemistry

Reseach and Professional Experience

Aug. 2002 – present — Executive Vice President of Research & Development, Metaproteomics, Research Laboratories, Ithaca, NY. Metaproteomics develops clinically proven, patented dietary supplements and pharmaceuticals from natural sources. Duties include the design and evaluation of experiments elucidating mechanism of action and biological activity within complex mixtures.

1998 – present — (5% Effort) National Coordinator for the USDA Minor Species Drug Program (NRSP-7). The NRSP-7 program is funded by the USDA to provide funds and expertise necessary for the approval of pharmaceuticals used in the treatment of diseases associated with minor crop species. Duties include the coordination of industrial, academic and regulatory resources necessary for protocol development through final drug approval.

1997 – present — Co-founder and Chairperson of BIOnexus, Ltd. Ithaca, NY. BIOnexus develops and markets nutritional supplements to address health problems associated with AIDS. NutriVir™, the BIOnexus supplement for wasting in HIV/AIDS, generated approximately $600,000 in gross revenues in its first year of sales. NutriVir™ is reimbursed by Medicaid in 14 states.

1991 – 1996	Founder, Chairperson, President and CEO of Paracelsian, Inc., Ithaca, NY. The Company was launched from the technology transfer program of Cornell University in 1991, and with the public offering in 1992 (Nasdaq,PRLN), became the first public corporation of a Cornell University technology transfer effort. Babish was associated with the attainment of over $12 million dollars in corporate financing.
1984 – 1996	Tenured, Associate and Professor of Pharmacology and Toxicology, Department of Pharmacology, College of Veterinary Medicine, Cornell University. Offered the first course in molecular risk assessment in the USA in 1979; member of the graduate Fields of Pharmacology, Toxicology, Veterinary Medicine, Food Science and Epidemiology; successfully petitioned the State of New York for the approval of the separate Fields of Toxicology and Pharmacology at Cornell University.
1978 – 1984	Assistant Professor, Department of Preventive Medicine, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY.
1976 – 1978	Postdoctoral Scientist, Food and Drug Research Labs, Waverly, NY.

Invited Presentations (Recent of 38 presentations)

Micronutrient deficiencies in AIDS wasting at Progressive Management of AIDS Wasting: 2000. Hunter College, NYC. March 24, 2000.

Phytochemicals and NF-kB activation at IBC's Conference on The Health Benefits of Natural Phytoceuticals. Montreal Bonaventure Hilton, July 22 – 23, 1997.

Chemically-induced cell cycle stasis in immunotoxicology. 12th Annual NIOSH Conference on Mechanisms of Immunotoxicology – Role of Apoptosis in Immunotoxicology. University of West Virginia, Morgantown, WV. September 10 – 12, 1997.

Publications (Selected of 108 peer-reviewed publications)

Payne M.A., Babish J.G., Bulgin M., Lane M., Wetzlich S., Craigmill A.L. (2002) Serum pharmacokinetics and tissue and milk residues of oxytetracycline in goats following a single intramuscular injection of a long-acting preparation and milk residues following a single subcutaneous injection. J Vet Pharmacol Ther. 25(1):25-32.

Calabrese C., Berman S.H., Babish J.G., et al. (2000) A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother Res. 14(5):333-338.

Ma,X., Stoffregen,D.A., Wheelock,G.D., Rininger,J.A. and Babish,J.G. (1997) Discordant hepatic expression of the cell division control enzyme p34cdc2 kinase, proliferating cell nuclear antigen, p53 tumor suppressor protein, and p21Waf1 cyclin-

dependent kinase inhibitory protein after WY14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid) dosing to rats. Mol. Pharmacol., 51, 69-78.

Rininger,J.A., Goldsworthy,T.L. and Babish,J.G. (1997) Time course comparison of cell-cycle protein expression following partial hepatectomy and WY14,643-induced hepatic cell proliferation in F344 rats. Carcinogenesis, 18, 935-941.

Rininger,J.A., Stoffregen,D.A. and Babish,J.G. (1997) Murine hepatic p53, RB, and CDK inhibitory protein expression following acute 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure. Chemosphere, 34, 1557-1568.

Rininger,J.A., Wheelock,G.D., Ma,X. and Babish,J.G. (1996) Discordant expression of the cyclin-dependent kinases and cyclins in rat liver following acute administration of the hepatocarcinogen [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (WY14,643). Biochem. Pharmacol., 52, 1749-1755.

Vancutsem,P.M. and Babish,J.G. (1996) In vitro and in vivo study of the effects of enrofloxacin on hepatic cytochrome P-450. Potential for drug interactions. Vet. Hum. Toxicol., 38, 254-259.

Patents (Selected of 15 US and three foreign patents)

US Patent No. **5,833,994**	11/10/1998 Use of the Ah receptor and Ah receptor ligands to treat or prevent cytopathicity of viral infection.
US Patent No. **5,612,188**	3/18/1997 Automated, multicompartmental cell culture system.
US Patent No. **5,529,899**	6/25/1996 Immunoassay for Ah receptor transformed by dioxin-like compounds.
US Patent No. **5,496,703**	3/5/1996 Indirect immunoassay for dioxin-like compounds

Exhibit B

LABORATORY NOTEBOOK SHEETS DOCUMENTING RESEARCH ON RIAA, IAA AND OTHER HOPS DERIVATIVES

Date	Notebook Number	Pages
6/4/02 - 6/24/02	2002-03	13 - 23
8/28/02	2002-04	3 - 4
12/5/02	2002-06	1 - 2
3/5/03	2002-06	43 - 44
4/23/03	2003-01	23
6/5/03	2003-01	45
9/3/03	2003-4	22
12/15/03	2003-5	42

PROJECT PGE2 Assay in RAW cells

Notebook No. 2002-03

13

Continued From Page

Experiment 2002-03-13

The general purpose + Procedure can be found on pages 4, 5, and 11 of this notebook

1_COX-2RAW_6.04.02

Compounds for Metagenics
PGE2 assay, using 1:10 dilution from the plates
6.04.02 [USING RAW 264.7 cells with LPS stimulation and 15 min with ARA]

Compound	Function	d1 ug/mL	d2 ug/mL	d3 ug/mL	d4 ug/mL	No. Wells
1. BetaTech - Alpha hop (10ug/uL)	Alpha hop =	50.000	10.000	5.000	1.000	8
2. BetaTech - Beta acid solution (10ug/uL)	Beta acid solution =	50.000	10.000	5.000	1.000	8
3. BetaTech Aromahop OE (10ug/uL)	Aromahop OE =	50.000	10.000	5.000	1.000	8
4. BetaTech Isohop (IAA) (10ug/uL)	Isohop (IAA) =	50.000	10.000	5.000	1.000	8
5. BetaTech Redhop (RIAA) (10ug/uL)	Redhop (RIAA) =	50.000	10.000	5.000	1.000	8
6. BetaTech Tetrahop Gold (10ug/uL)	Tetrahop Gold =	50.000	10.000	5.000	1.000	8
7. BetaTech Hexahop gold (HHIAA) (10ug/uL)	Hexahop gold =	50.000	10.000	5.000	1.000	8
8. BetaTech Hop oil (10ug/uL)	Hop oil =	50.000	10.000	5.000	1.000	8
9. Diisopropyl fluorophosphate	DFP =	50.000	5.000	0.500	0.050	8
10. Inflavanoid IC Tablets	Inflavanoid IC =	50.000	25.000	12.500	6.250 total =	8 80

2_COX-2RAW_6.04.02

New Hops Compounds for Metagenics
PGE2 assay, using 1:10 dilution from the plates
6.04.02 [USING RAW 264.7 cells with LPS stimulation and 15 min with ARA]

Compound	Function	d1 ug/mL	d2 ug/mL	d3 ug/mL	d4 ug/mL	No. Wells
1. BetaTech Redhop (RIAA) (10 ug/uL)	RIAA =	50.000	10.000	5.000	1.000	8
2. RIAA:ursolic acid (1:1)	Total =	50.000	10.000	5.000	1.000	8
	RIAA =	25.000	5.000	2.500	0.500	
	Ursolic acid =	25.000	5.000	2.500	0.500	
3. Lemon Bioflavonoid (RM07782)	Bioflavonoid =	50.000	10.000	5.000	1.000	8
4. Ginger (RM07782)	Ginger =	50.000	10.000	5.000	1.000	8
5. RIAA:curcumin granular (60026) (1:1)	Total =	50.000	10.000	5.000	1.000	8
	RIAA =	25.000	5.000	2.500	0.500	
	Curcumin =	25.000	5.000	2.500	0.500	
6. Cayenne Pepper (RM06972)	Cayenne =	50.000	10.000	5.000	1.000	8
7. RIAA:Quercetin (07971) (1:1)	Total =	50.000	10.000	5.000	1.000	8
	RIAA =	25.000	5.000	2.500	0.500	
	Quercetin =	25.000	5.000	2.500	0.500	
8. RIAA:Alpha galanga (1:1)	Total =	50.000	10.000	5.000	1.000	8
	RIAA =	25.000	5.000	2.500	0.500	
	Alpha galanga =	25.000	5.000	2.500	0.500	
9. RIAA:Boswellin (RM07781) (1:1)	Total =	50.000	10.000	5.000	1.000	8
	RIAA =	25.000	5.000	2.500	0.500	
	Boswellin =	25.000	5.000	2.500	0.500	
10. Nimesulide	Nimesulide =	50.000	10.000	5.000	1.000 total =	8 80

Continued on Page 14

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Signed Date

Signed Date

6.4.02 7-19-02

PROJECT PGE, Assay in 24 wells

Notebook No. 2002-03

Continued From Page 13

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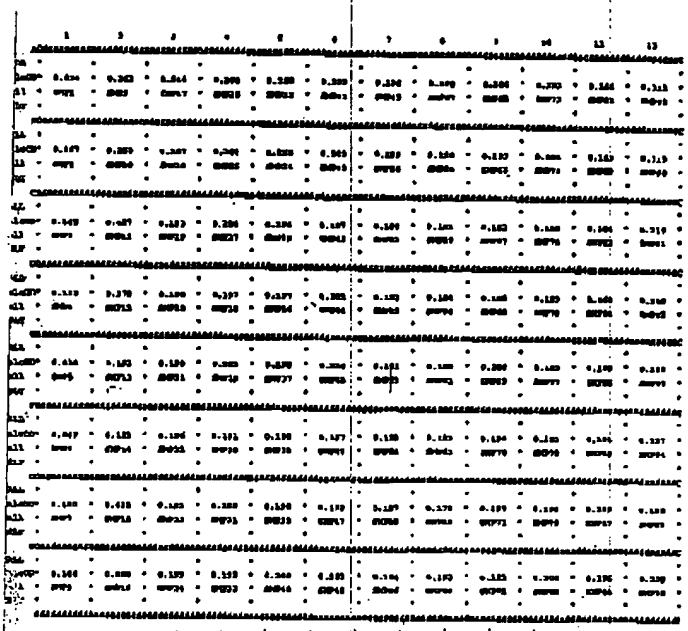
2002-03-13 cont

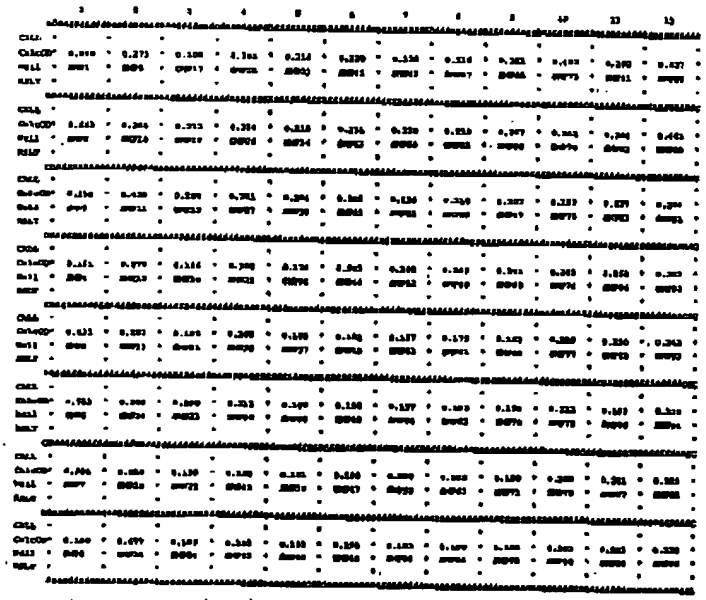
Bio-Tek Instruments

Assay: Quick Read
Wavelength: 405
Date: 06/06/02
Time: 02:46:03PM
Temp:
Lot: Plate 1 PGE
Operator:
Plate ID:



Bio-Tek Instruments

Assay: Quick Read
Wavelength: 405
Date: 06/06/02
Time: 02:46:52PM
Temp:
Lot: Plate 2 PGE
Operator:
Plate ID:



Continued on Page 15

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Signed — Date: 6-6-02

Signed — Date: 7-19-02

PROJECT PGE2 Assay in RAW cells

Notebook No. 2002-03

Continued From Page 14

15

2002-03-13 cont.

3_COX-DRAW_8.04.02

New Hope Compounds for Metagenics
PGE2 assay, using 1:10 dilution from the plates
8.04.02 [URBIG RAW 264.7 cells with LPS stimulation and 15 min with ARA]

1. Aspirin A-9376 Lot 119H0475 1ml	Aspirin =	50.000	10.000	5.000	1.000
2. Aspirin/asch (1:1) Acetyl Acid N-4124 Lot 71K0442 1ml	Total =	50.000	10.000	5.000	1.000
	Aspirin =	25.000	5.000	2.500	0.500
	Asch =	25.000	5.000	2.500	0.500
3. AH86 - Lipotech A1057 640ml	AH86 =	50.000	10.000	5.000	1.000
4. Hesperidin - Metagenics (07791) 640ml	Hesperidin =	50.000	10.000	5.000	1.000
5. Ascorbic acid - Metagenics (RM074493) ANO916 640ml	Ascorbic acid =	50.000	10.000	5.000	1.000
6. RIAA/oleanolic acid (1:1) O-5604 Lot 60K5234 400ml	Total =	50.000	10.000	5.000	1.000
	RIAA =	25.000	5.000	2.500	0.500
	oleanolic acid =	25.000	5.000	2.500	0.500
7. A70 - Lipotech AN128 480ml	A70 =	50.000	10.000	5.000	1.000
8. Garlic -SuperGarlic 6000 - Metagenics AN118 720ml	Garlic =	50.000	10.000	5.000	1.000
9. curcumin granular (08656)/ursolic acid (1:1)	Total =	50.000	10.000	5.000	1.000
	Curcumin =	25.000	5.000	2.500	0.500
	Ursolic acid =	25.000	5.000	2.500	0.500
10. curcumin granular (08656)/oleanolic (1:1)	Total =	50.000	10.000	5.000	1.000
	Curcumin =	25.000	5.000	2.500	0.500
	Oleanolic acid =	25.000	5.000	2.500	0.500
					total = 80

1:2 dilutions

Bio-Tek Instruments

Assay: Quick Read
Wavelength:405
Comments:

Date:08/07/02
Time:02:26:52PM
Temp:

Lot: Plate 1
Operator:
Plate ID:

This plate was done with a 1:50 dilution for PGE2 in the media.

Continued on Page

Read and Understood By

Signed — Date 6.7.02

Signed — Date 7-19-02

PROJECT PGE2 In RAW Cells

Notebook No 2002-03

Continued From Page

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Experiment 2002-03-16

The purpose and procedure for this experiment can be found on pages 4, 8, and 11 of this notebook.

3_COX-2RAW_6.10.02

Compounds for Mutagenics
PGE2 assay, using 1:10 dilution from the plates
6.10.02 [USING RAW 264.7 cells with LPS stimulation and 15 min with ARA] Dilute media 1:50 and 1:100 for PGE2 assay

1. BetaTech - Alpha hop (10µg/µL)	Alpha hop =	50.000	10.000	5.000	1.000	8
2. BetaTech - Beta acid solution (10µg/µL)	Beta acid solution =	50.000	10.000	5.000	1.000	8
3. BetaTech Aromahop OE (10µg/µL)	Aromahop OE =	50.000	10.000	5.000	1.000	8
4. BetaTech Isohop (IAA) (10µg/µL)	Isohop (IAA) =	50.000	10.000	5.000	1.000	8
5. BetaTech Redhop (RIAA) (10µg/µL)	Redhop (RIAA) =	50.000	10.000	5.000	1.000	8
6. BetaTech Tetrahop Gold (10µg/µL)	Tetrahop Gold =	50.000	10.000	5.000	1.000	8
7. BetaTech Hexahop gold (HHIAA) (10µg/µL)	Hexahop gold =	50.000	10.000	5.000	1.000	8
8. Ibuprofen	Ibuprofen =	50.000	10.000	5.000	1.000	8
9. Celebrex	Celebrex =	5.000	0.500	0.050	0.005	8
10. Indevanold IC	Indevanold IC =	50.000	10.000	5.000	1.000 total =	8 80

BIO-TEK MICROPLATE READER 06/14/02 AT 01:02 PM 80011864

ASSAY#

PLATE# OPERATOR

NOTES

PROGRAM MODE #9 SINGLE WAVELENGTH: 405

1:50

TABLE OF ABSORBANCE VALUES

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.887	0.450	0.391	0.341	0.376	0.317	0.329	0.346	0.342	0.697	0.667	0.678
B	1.828	0.333	0.386	0.329	0.371	0.335	0.321	0.326	0.344	0.685	0.689	0.784
C	0.193	0.666	0.519	0.382	0.479	0.315	0.334	0.348	0.384	0.397	0.662	0.698
D	0.163	0.637	0.535	0.314	0.516	0.531	0.527	0.565	0.521	0.588	0.659	0.388
E	0.568	0.578	0.599	0.577	0.562	0.581	0.576	0.616	0.638	0.615	0.649	0.573
F	0.667	0.586	0.514	0.513	0.533	0.554	0.537	0.536	0.526	0.552	0.648	0.554
G	0.156	0.673	0.523	0.532	0.544	0.546	0.568	0.549	0.535	0.596	0.604	0.682
H	0.165	0.655	0.519	0.573	0.519	0.547	0.517	0.548	0.546	0.531	0.638	0.534

BIO-TEK MICROPLATE READER 06/14/02 AT 01:05 PM 80011865

ASSAY#

PLATE# OPERATOR

NOTES

PROGRAM MODE #9 SINGLE WAVELENGTH: 405

1:100

TABLE OF ABSORBANCE VALUES

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.988	0.641	0.682	0.681	0.639	0.999	0.628	0.621	0.627	0.788	0.694	0.728
B	1.881	0.620	0.559	0.578	0.628	0.682	0.594	0.686	0.624	0.698	0.782	0.732
C	0.169	0.639	0.566	0.535	0.531	0.553	0.586	0.578	0.598	0.639	0.664	0.657
D	0.163	0.627	0.584	0.551	0.572	0.577	0.583	0.606	0.598	0.612	0.678	0.633
E	0.678	0.497	0.635	0.612	0.617	0.648	0.625	0.626	0.598	0.649	0.663	0.661
F	0.595	0.596	0.589	0.585	0.564	0.588	0.577	0.609	0.602	0.628	0.658	0.622
G	0.154	0.673	0.625	0.598	0.588	0.575	0.596	0.594	0.638	0.631	0.783	0.662
H	0.152	0.680	0.646	0.648	0.628	0.631	0.638	0.635	0.782	0.616	0.783	0.628

The same plate was run @ 2 different dilutions. 1:50 and 1:100

Continued on Page

Read and Understood By

Signed 6.14.02 Date

Signed 7-19-02 Date

PROJECT PGE_2 Assay + NO Assay

Notebook No. 2002-03

Continued From Page

Experiment 2002-03-17

- The general purpose and procedure for the PGE_2 Assay can be found on 2002-03-01 experiment.

- Purpose for the NO experiment to determine the levels of Nitrite Oxide being produce in the cells.

- Procedure for NO.

Griess Reagent was made.

1% - Sulfanilamide Sigma S-9251 lot 101K2010
0.1% - N-[Naphthyle]ethylenediamine (Sigma N-5889) lot 71K0112
2.5% - H_3PO_4 (Riedel-de Haën -0410) Lot 52540

This reagent is mixed up in Ultra Pure water

25 mls = 250mg Sulfanilamide
25mg N-[Naphthyle]ethylenediamine
625mg H_3PO_4

The same plates of cells were used for each experiment.

The cells were taken from 1xT75 plate of RAW cells that were 80% confluent. and plated into 2 96 well plates at 4×10^5 cells per well.

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Read and Understood By

Signed 6-17-02 Date

Signed 7-19-02 Date

PROJECT PGE2 + NO in RAW cells

Notebook No. 20023

Continued From Page 17

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2002-03-pg17 cont.

1_COX-2RAW_6.17.02

Compounds for Metagenics
PGE2 assay, using 1:1 and 1:10 dilution from the plates
6.17.02 [USING RAW 264.7 cells with LPS stimulation and 15 min with 5 mM ARA]

Compound		C1	C2	C3	C4	No. Wells
1. BetaTech - Alpha hop (10µg/µL)	Alpha hop =	50.000	10.000	5.000	1.000	8
2. BetaTech - Beta acid solution (10µg/µL)	Beta acid solution =	50.000	10.000	5.000	1.000	8
3. BetaTech Aromahop OE (10µg/µL)	Aromahop OE =	50.000	10.000	5.000	1.000	8
4. BetaTech Isohop (IAA) (10µg/µL)	Isohop (IAA) =	50.000	10.000	5.000	1.000	8
5. BetaTech Redihop (RIAA) (10µg/µL)	Redihop (RIAA) =	50.000	10.000	5.000	1.000	8
6. BetaTech Tetrahop Gold (10µg/µL)	Tetrahop Gold =	50.000	10.000	5.000	1.000	8
7. BetaTech Hexahop gold (HHIAA) (10µg/µL)	Hexahop gold =	50.000	10.000	5.000	1.000	8
8. Ibuprofen	Ibuprofen =	50.000	10.000	5.000	1.000	8
9. Celebrex	Celebrex =	5.000	0.500	0.050	0.005	8
10. Inflavanoid IC Tablets	Inflavanoid IC =	50.000	10.000	5.000	1.000 total =	8 80

2_COX-2RAW_6.17.02

Compounds for Metagenics
PGE2 assay, using undiluted and 1:1 dilution from the plates
6.17.02 [USING RAW 264.7 cells no LPS stimulation and 15 min with 5 µM ARA]

Compound		C1	C2	C3	C4	No. Wells
1. BetaTech - Alpha hop (10µg/µL)	Alpha hop =	50.000	10.000	5.000	1.000	8
2. BetaTech - Beta acid solution (10µg/µL)	Beta acid solution =	50.000	10.000	5.000	1.000	8
3. BetaTech Aromahop OE (10µg/µL)	Aromahop OE =	50.000	10.000	5.000	1.000	8
4. BetaTech Isohop (IAA) (10µg/µL)	Isohop (IAA) =	50.000	10.000	5.000	1.000	8
5. BetaTech Redihop (RIAA) (10µg/µL)	Redihop (RIAA) =	50.000	10.000	5.000	1.000	8
6. BetaTech Tetrahop Gold (10µg/µL)	Tetrahop Gold =	50.000	10.000	5.000	1.000	8
7. BetaTech Hexahop gold (HHIAA) (10µg/µL)	Hexahop gold =	50.000	10.000	5.000	1.000	8
8. Ibuprofen	Ibuprofen =	50.000	10.000	5.000	1.000	8
9. Celebrex	Celebrex =	5.000	0.500	0.050	0.005	8
10. Inflavanoid IC Tablets	Inflavanoid IC =	50.000	10.000	5.000	1.000 total =	8 80

The compounds used on these two plates are identical. The amount of Arachidonic Acid used is not.

4 Plates will be used for PGE2 and Three for NO.

1- Alpha hop - AV1124
2- Beta Acid - AV1125
3- Aroma Hop - AV1126
4- Isohop - AV1127
5- Redihop - AV1128
6- Tetrahop - AV1129
7- Hexahop - AV1130
8- Ibuprofen Sigma I-4883 Lot-26H1368
9- Celebrex - AV1055
10- Inflavanoid IC Tablets - AV1101

Continued on Page 19

Signed

6.19.02
6.18.02
Date

Read and Understood By

Signed

7-19-02
Date

PROJECT PGE$_2$ + NO Assay in RAW cells. Notebook No. 2002-03-TH 19

Continued From Page 18

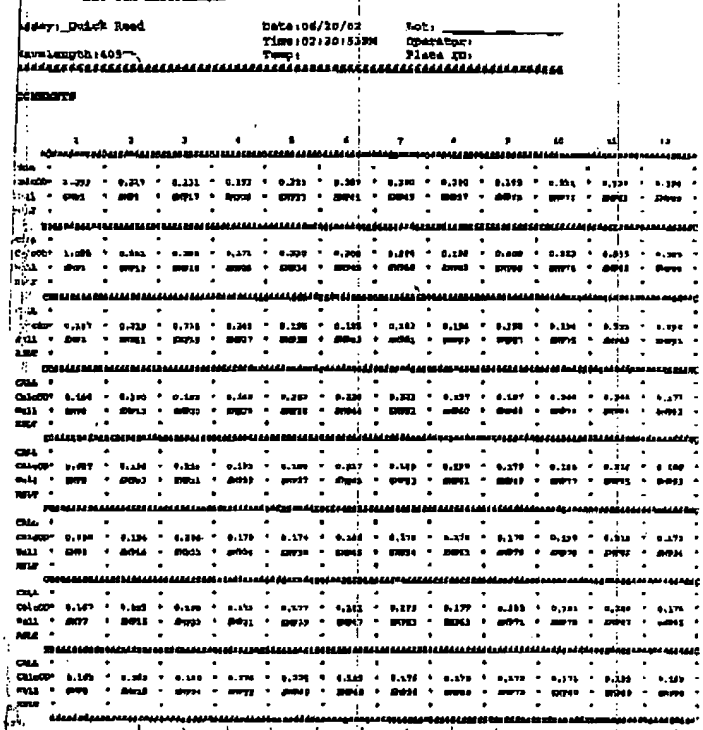
Experiment 2002-03-17 cont.

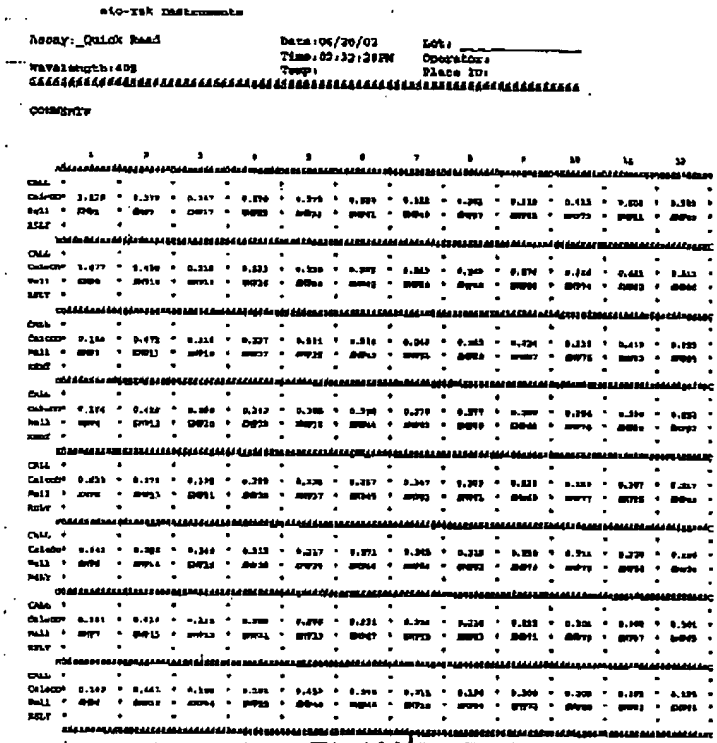
~~The NO plate were~~ as TH

The Plates were treated and supernate collected for the PGE$_2$ Plates. Then the Plates were put back into the incubator overnight.

COX-2 1:2

COX-2 1:10





This is the raw data for the COX-2 plate diluted 1:2

This is the RAW data for the COX-2 plate diluted 1:10

Continued on Page 20

Read and Understood By

Signed 6.20.02 Date

Signed 7-19-02 Date

PROJECT PGE$_2$ + NO on RAW Cells

Notebook No. 2002-03

Continued From Page 19

Experiment 2002-03-19 cont.

COX-1 1:2

Bio-Tek Instruments

Assay: Quick Read Date: 06/20/02 Lot:
Time: 02:35:42PM Operator:
Wavelength: 405 Temp: Plate ID:

Comments

This is the data from the RAW plate COX-1 1:2 dilution.

Bio-Tek Instruments

Assay: Quick Read Date: 06/20/02 Lot:
Time: 02:34:04PM Operator:
Wavelength: 405 Temp: Plate ID:

Comments

This is data from the Undiluted COX-1 plate.

Continued on Page 21

Read and Understood By

Signed 6-20-02 Date

Signed 7-19-02 Date

PROJECT PGE, +NO in RAW cells

Notebook No 2002-03

Continued From Page 20

Experiment 2002-03-17 cont.

A Standard Curve was done on the NO.

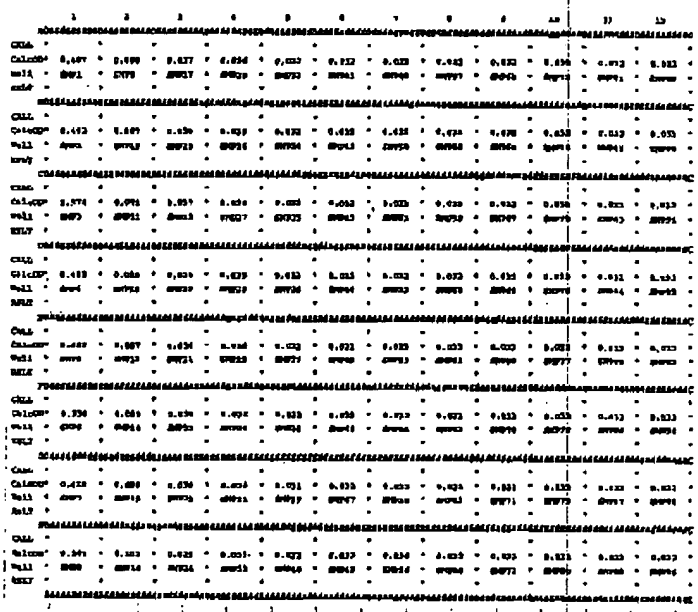
50ul of Media off of the treated cells was added to 50ul of the Griess reagent. This was allowed to sit for 10 minutes then read @ 605 nM.

No St. Curve

Bio-Tek Instruments

Assay: Quick Read Data: 06/20/02 Lot:
Time: 03:28:33 PM Operator:
Wavelength: 630 Temp: Plate ID:

COMMENTS



This is the NO Standard curve.

Continued on Page 22

Read and Understood By

Signed — Date 6-20-02

Signed — Date 7-19-02

PROJECT RPE2 - No Assay in RAW cells

Notebook No. 2002-03

Continued From Page 21

22

Experiment 2002-05-17

Bio-Tek Instruments

Assay: Quick Read Date: 05/20/02 Lot:
Wavelength: 405 Time: 02:35:40PM Operator:
Temp: Plate ID:

COMMENTS

COX-2 RAW data NO

Bio-Tek Instruments

Assay: Quick Read Date: 05/20/02 Lot:
Wavelength: 405 Time: 02:36:07PM Operator:
Temp: Plate ID:

COMMENTS

COX-1 RAW data NO

Continued on Page

Read and Understood By

Signed 6-20-02 Date

Signed 7-19-02 Date

PROJECT PGE_2 in RAW cells + NO Assay

Notebook No. 2002-03

Continued From Page

Experiment: 2002-05-23

The purpose and procedure can be found on page 11 of this notebook.

1_COX-2RAW_6.24.02

Compounds for Metagenics
PGE2 assay, using 1:1 and 1:10 dilution from the plates
6.24.02 [USING RAW 264.7 cells with dLPS stimulation and 15 min with 8 uM ARA]

Compound		Conc. in DMSO	Conc. in Assay			No. Wells
1. Alcohol extract of spent hops	EtOH/SH =	50.000	5.000	0.500	0.050	8
2. Ursolic acid 90% - Sabinsa	Ursolic acid =	50.000	5.000	0.500	0.050	8
3. Carnosic acid (Rosemary)	Betulinic acid =	50.000	5.000	0.500	0.050	8
4. Oleanolic acid 60% - Sabinsa	Oleanolic acid 60% =	50.000	5.000	0.500	0.050	8
5. BetaTech Radhop (RIAA) (10µg/µL)	Radhop (RIAA) =	50.000	5.000	0.500	0.050	8
6. Curcumin granular (06856)	Curcumin =	50.000	5.000	0.500	0.050	8
7. RIAA:Ursolic acid (90%) - [1:1]	Total =	50.000	5.000	0.500	0.050	8
	RIAA =	25.000	2.500	0.250	0.025	
	Ursolic acid 90% =	25.000	2.500	0.250	0.025	
8. Ibuprofen - Sigma	Ibuprofen =	50.000	5.000	0.500	0.050	8
9. Celebrex	Celebrex =	5.000	0.500	0.050	0.005	8
10. Aspirin - Sigma	Aspirin =	50.000	10.000	5.000	1.000	8
					total =	80

2_COX-1RAW_6.24.02

Compounds for Metagenics
PGE2 assay, using undiluted and 1:1 dilution from the plates
6.24.02 [USING RAW 264.7 cells no LPS stimulation and 60 min with 100 µM ARA]

Compound		Conc. in DMSO	Conc. in Assay			No. Wells
1. Alcohol extract of spent hops		50.000	5.000	0.500	0.050	8
2. Ursolic acid 90% - Sabinsa		50.000	5.000	0.500	0.050	8
3. Carnosic acid (Rosemary)		50.000	5.000	0.500	0.050	8
4. Oleanolic acid 60% - Sabinsa		50.000	5.000	0.500	0.050	8
5. BetaTech Radhop (RIAA) (10µg/µL)	Radhop (RIAA) =	50.000	5.000	0.500	0.050	8
6. Curcumin granular (06856)		50.000	5.000	0.500	0.050	8
7. RIAA:Ursolic acid (90%) - [1:1]	Total =	50.000	5.000	0.500	0.050	8
	RIAA =	25.000	2.500	0.250	0.025	
	Ursolic acid 90% =	25.000	2.500	0.250	0.025	
8. Ibuprofen - Sigma	Ibuprofen =	50.000	5.000	0.500	0.050	8
9. Celebrex	Celebrex =	6.000	0.500	0.050	0.005	8
10. Aspirin - Sigma	Aspirin =	50.000	10.000	5.000	1.000	8
					total =	80

Continued on Page 24

Read and Understood By

Signed 6.24.02 Date

Signed 7-19-02 Date

PROJECT PGE2 Expression in A549 cells Notebook No. ~~2002-03~~ 2002-04 TH 3

Continued From Page 2

2002 ~~03~~ 04-01 cont.

C2_COX2_A549_8.25.02

A549 Cells - wash, cells treated with test material, stimulated with IL-1/LPS/H2O2 for 24 hrs and assayed for PGE2. For PGE2 assay run media undiluted and diluted 1:20. 8.26.02

1. BetaTech (IAA)	IAA =	25	5.0	0.5	0.05	8
2. BetaTech (RIAA)	BetaTech (RIAA) =	25	5.0	0.5	0.05	8
3. IAA:RIAA [1:1]	Total =	25	5.0	0.5	0.05	8
	IAA =	12.5	2.5	0.25	0.025	
	RIAA =	12.5	2.5	0.25	0.025	
4. IAA:RIAA - [2:1]	Total =	25	5	0.5	0.05	8
	IAA =	16.7	3.3	0.333	0.033	
	RIAA =	8.3	1.7	0.167	0.017	
5. IAA:RIAA - [5:1]	Total =	25	5	0.5	0.05	8
	IAA =	20.8	4.2	0.417	0.042	
	RIAA =	4.2	0.8	0.083	0.008	
6. IAA:RIAA - [10:1]	Total =	23	5	0.5	0.05	8
	IAA =	22.7	4.55	0.4545	0.0455	
	RIAA =	2.3	0.45	0.0455	0.0045	
7. IAA:RIAA - [50:1]	Total =	25	5	0.5	0.05	8
	IAA =	24.5	4.90	0.490	0.049	
	RIAA =	0.5	0.10	0.010	0.001	
8. IAA:RIAA - [100:1]	Total =	25	5	0.5	0.05	8
	IAA =	24.75	4.95	0.4950	0.0495	
	RIAA =	0.25	0.05	0.0050	0.0005	
9. IAA:Tryptanthrin - [1:1]	Total =	25	5	0.5	0.05	8
	IAA =	12.5	2.5	0.25	0.025	
	Tryptanthrin =	12.5	2.5	0.25	0.025	
					Total =	72

Five 1000, 500, 16.5 and 7.8 concentrations of the standard curve as series in Column 2

same compounds as on page 2 of this notebook

This plate was treated the same as the plate on page 2 of this notebook

C3_COX2_A549_8.25.02&TDS

A549 Cells - cells are stimulated with IL-1/LPS/H2O2 for 24 hrs, washed, test materials added for 60 minutes then A23187 [50 µM] added as assay for PGE2 30 minutes later. For PGE2 assay run media undiluted and 1:20 8.26.02

1. Diisopropyl fluorophosphate	COX-2	25	5.0	0.5	0.05	8
2. Vioxx	COX-2	25	5.0	0.5	0.05	8
3. Celebrex	COX-2	25	5.0	0.5	0.05	8
4. Nimesulide	COX-2	25	5.0	0.5	0.05	8
5. Ibuprofen	COX-2/COX-1	25	5.0	0.5	0.05	8
6. Indomethacin	COX-1	25	5.0	0.5	0.05	8
7. Aspirin	COX-1	25	5.0	0.5	0.05	8
8. Salicylic acid	-	25	5.0	0.5	0.05	8
9. Naproxen	-	25	5.0	0.5	0.05	8
10. Acetaminophen	-	25	5.0	0.5	0.05	8
					Total =	80

1- Aldrich, D12,600-4 Lot 02456325
2- AN10066
3- AN10055
4- N-1016 lot 117H1019
5- Sigma I-4663 lot 76H1368
6- Sigma I-7378 lot 60K0745
7- A-5376 lot 119H0175
8- AN10071
9- N-8280 lot 11K1767
10- A-2085 lot 20K0648
A- 23187 - Sigma C-7522 lot 81K4002

This plate was stimulated with IL-1/LPS and H2O2 for 24 hrs. The plate will then be washed and test materials added for 1hr then stimulated with A23187 for 30 minutes

Continued on Page 4

Read and Understood By

Signed 8.29.02 Date

Signed 9-1-02 Date

PROJECT PGE2 Expression In A-549 & AGS Cells **Notebook No.** 2002-04

Continued From Page 3

Experiment 2002-04-01

AGS Cells Format A - wash, add test material and assay for PGE2 the next day
For PGE2 assay run media undiluted
8.26.02

1. Oleanolic acid (90% Sabinsa)	Oleanolic acid =	25	5.0	0.5	0.05	8
2. BetaTech (RIAA)	BetaTech (RIAA) =	25	5.0	0.5	0.05	8
3. Tryptanthrin - Wako Chemicals	Tryptanthrin =	10	1	0.1	0.01	8
4. RIAA:Oleanolic acid - [10:1]	Total =	25	5	0.5	0.05	8
	RIAA =	22.7	4.5	0.455	0.045	
	Oleanolic =	2.3	0.5	0.045	0.005	
5. RIAA:Oleanolic acid - [5:1]	Total =	25	5	0.5	0.05	8
	RIAA =	20.8	4.2	0.417	0.042	
	Oleanolic =	4.2	0.8	0.083	0.008	
6. RIAA:Oleanolic acid - [1:5]	Total =	25	5	0.5	0.05	8
	RIAA =					
	Oleanolic =					
7. RIAA:Oleanolic acid - [1:10]	Total =	25	5	0.5	0.05	8
	RIAA =					
	Oleanolic =					
8. 81115 Metogenics	81115 =	25	5	0.5	0.05	8
9. RIAA:Tryptanthrin - [1:1]	Total =	25	5	0.5	0.05	8
	RIAA =					
	Tryptanthrin =					
					Total =	72

Run 1000, 500, 15.6 and 7.8 concentrations of the standard curve as series in Column 2

AGS Cells Format A - wash, add test material and assay for PGE2 the next day
For PGE2 assay run media undiluted
8.26.02

4. BetaTech (IAA)	IAA =	25	5.0	0.5	0.05	8
5. BetaTech (RIAA)	BetaTech (RIAA) =	25	5.0	0.5	0.05	8
3. IAA:RIAA [1:1]	Total =	25	5.0	0.5	0.05	8
	IAA =	12.5	2.5	0.25	0.025	
	RIAA =	12.5	2.5	0.25	0.025	
4. IAA:RIAA - [2:1]	Total =	25	6	0.6	0.05	8
	IAA =	16.7	3.3	0.333	0.033	
	RIAA =	8.3	1.7	0.167	0.017	
5. IAA:RIAA - [5:1]	Total =	25	5	0.5	0.05	8
	IAA =	20.8	4.2	0.417	0.042	
	RIAA =	4.2	0.8	0.083	0.008	
6. IAA:RIAA - [10:1]	Total =	25	5	0.5	0.05	8
	IAA =	22.7	4.55	0.4545	0.0455	
	RIAA =	2.3	0.45	0.0455	0.0045	
7. IAA:RIAA - [50:1]	Total =	25	5	0.5	0.05	8
	IAA =	24.5	4.90	0.490	0.049	
	RIAA =	0.5	0.10	0.010	0.001	
8. IAA:RIAA - [100:1]	Total =	25	5	0.5	0.05	8
	IAA =	24.75	4.95	0.4950	0.0495	
	RIAA =	0.25	0.05	0.0050	0.0005	
9. IAA:Tryptanthrin - [1:1]	Total =	25	5	0.5	0.05	8
	IAA =	12.5	2.5	0.25	0.025	
	Tryptanthrin =	12.5	2.5	0.25	0.025	
					Total =	72

Run 1000, 500, 15.6 and 7.8 concentrations of the standard curve as series in Column 2

AGS Cells - cells grown to confluence, wash, test materials added for 60 minutes then A23187 [10 µM] added and assay for PGE2 30 minutes later.
For PGE2 assay run media undiluted and 1:20
8.26.02

1. Diisopropyl fluorophosphate	COX-2	25	5.0	0.5	0.05	8
2. Vioxx	COX-2	25	5.0	0.5	0.05	8
3. Celebrex	COX-2	25	5.0	0.5	0.05	8
4. Nimesulide	COX-2	25	5.0	0.5	0.05	8
5. Ibuprofen	COX-2/COX-1	25	5.0	0.5	0.05	8
6. Indomethacin	COX-1	25	5.0	0.5	0.05	8
7. Aspirin	COX-1	25	5.0	0.5	0.05	8
8. Salicylic acid	-	25	5.0	0.5	0.05	8
9. Naproxen	-	25	5.0	0.5	0.05	8
10. Acetaminophen	-	25	5.0	0.5	0.05	8
					Total =	80

The first two plates were run in the exact same manner as the A-549 plates outlined on pages 2 and three of this notebook

Plate 3 was done with the test materials added and then 60 minutes later they were stimulated w/ A23187 for 30 minutes before supes were taken for PGE2 plates

Continued on Page 5

Read and Understood By

Signed [signature] Date 8-28-02

Signed [signature] Date 9-1-02

PROJECT Cell Lysate and Cell of U-937

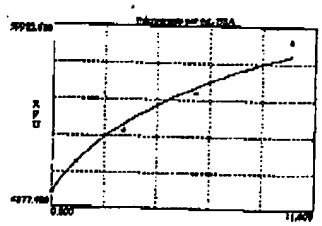
Notebook No. 2002-06

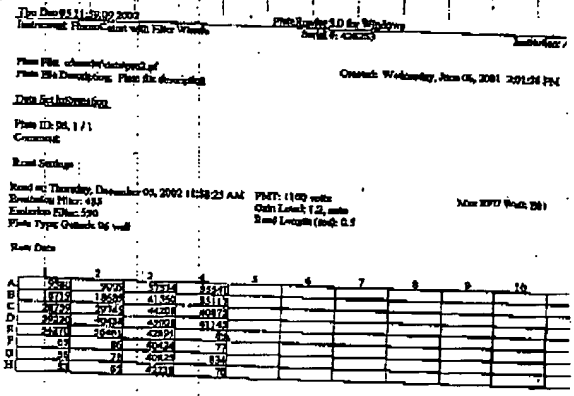
Continued From Page

Experiment 2002-06-01

Purpose to look @ U-937 cells with VD3 by RIAA.

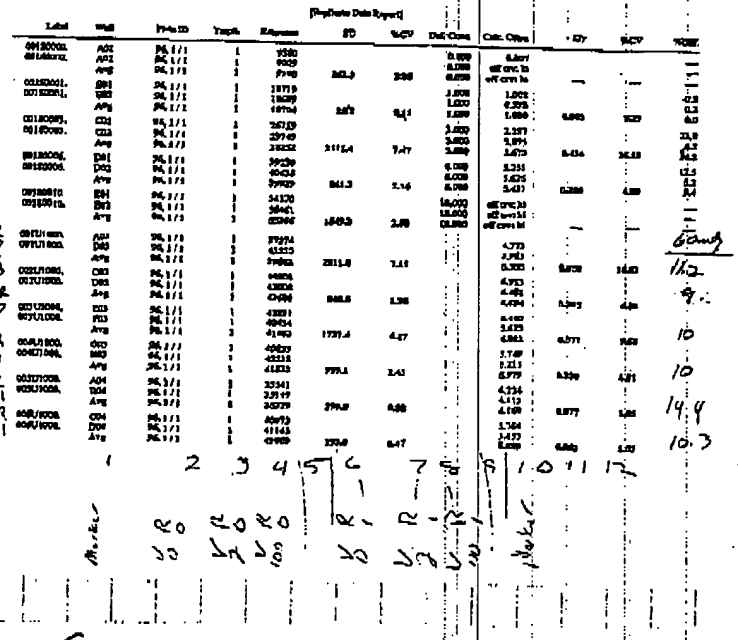
Protein Assay





Thursday, December 05, 2002

I-Smart



Continued on Page 2

Read and Understood By

Signed 12-5-02 Date

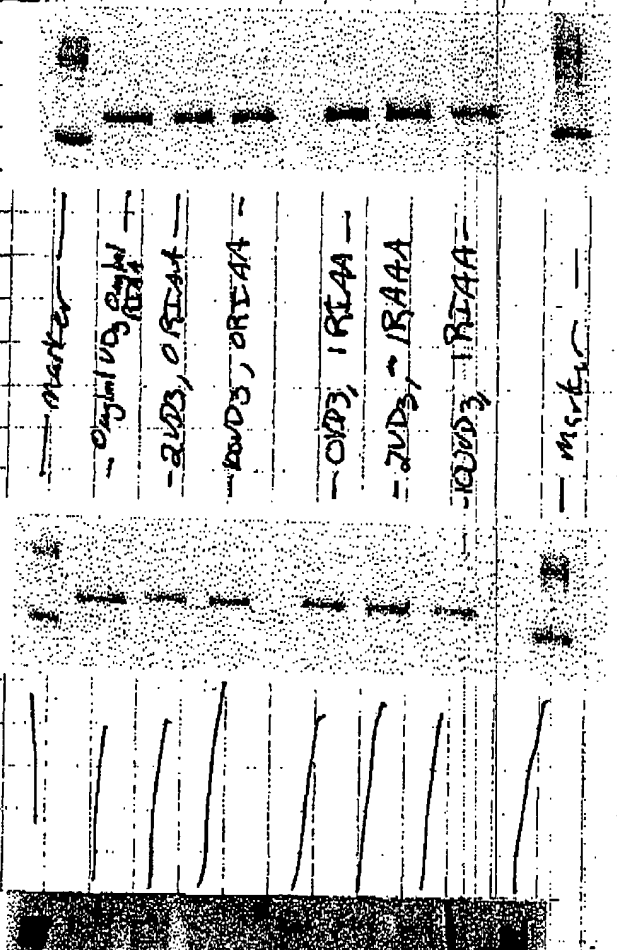
Signed 12-25-02 Date

PROJECT Cell Lysate + Gels of PUS 37

Notebook No 2002-06

Continued From Page 1

Experiment 20002-06-01



COX-2 - 56KD

COX-1 66KD

iNOS 130KD

Continued on Page

Read and Understood By

Signed

12-5-02

Date

Signed

12-23-02

Date

PROJECT PGE Assay in AGS cells

Notebook No. 2002-06

Continued From Page

43

Experiment 2002-06-43

Purpose + Procedure can be found on p. 585

2002-06-11

C1_COXAGS_03.03.03

AGS Cells - cells grown to confluence, wash, test materials added for 60 minutes then A23187 [50 µM] added and assay for PGE2 30 minutes later.
For PGE2 assay run media diluted 1:20 only
3.03.03

Compound	Comments	d1 [µg/mL]	d2 [µg/mL]	d3 [µg/mL]	d4 [µg/mL]	No Wells
1. Ultrainflammax Ingredients		50	5.0	0.5	0.05	8
2. META551		50	5.0	0.5	0.05	8
3 Rosemary extract (07720)		50	5.0	0.5	0.05	8
4. Oleanolic acid (80% Sabinsa)		50	5.0	0.5	0.05	8
5. RIAA (Haas)		50	5.0	0.5	0.05	8
6. Rutin (06394)		50	5.0	0.5	0.05	8
7. Curcumin (07367)		50	5.0	0.5	0.05	8
8. Ginger root (06836)		50	5.0	0.5	0.05	8
9. AH88		50	5.0	0.5	0.05	8
10. Aspirin (Sigma)		50	5.0	0.5	0.05	8
					Total =	80

C2_COXAGS_03.03.03

AGS Cells - cells grown to confluence, wash, test materials added for 60 minutes then A23187 [50 µM] added and assay for PGE2 30 minutes later.
For PGE2 assay run media diluted 1:20 only
3.03.03

Compound	Comments	d1 [µg/mL]	d2 [µg/mL]	d3 [µg/mL]	d4 [µg/mL]	No Wells
1. Ischop AN1127		50	5.0	0.5	0.05	8
2. #1115		50	5.0	0.5	0.05	8
3. Tetrahop AN1120		50	5.0	0.5	0.05	8
4. Hexahop AN1130		50	5.0	0.5	0.05	8
5. Alphahop AN1124		50	5.0	0.5	0.05	8
6. BetaStab AN1125		50	5.0	0.5	0.05	8
7. Iso-Rich AN1080		50	5.0	0.5	0.05	8
8. Tamrin Extract #4411 AN1173		50	5.0	0.5	0.05	8
9. A70 LIPOTECH		50	5.0	0.5	0.05	8
10. Aromahop AN1126		50	5.0	0.5	0.05	8
					Total =	80

Continued on Page 44

Read and Understood By

Signed 3-5-03 Date

Signed 3-9-03 Date

PROJECT PGE2 Assay in ASB cells

Notebook No. 2002-05

Continued From Page 43

Experiment 2002-6-03 cont'd

Raw Data.

Bio-Tek Instruments

Assay: Quick Read

Date: 03/07/03

Time: 12:47:57PM

Lot: AG 5 1-70 Plate 1

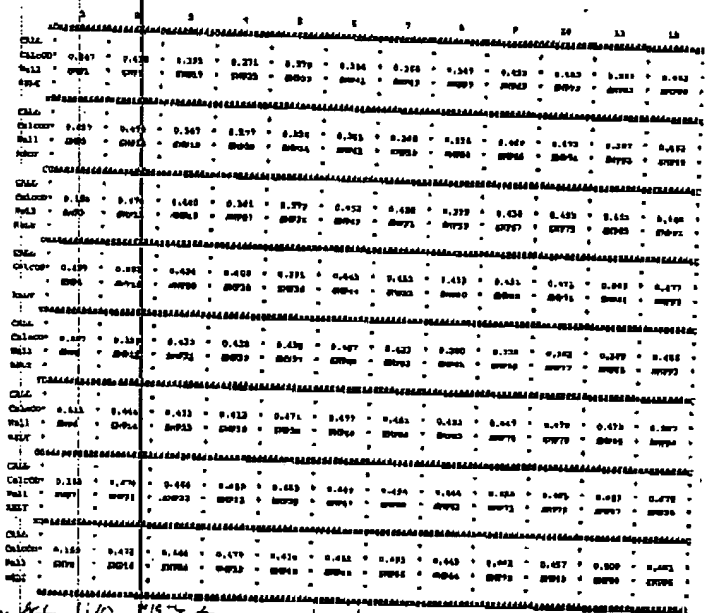
Operator:

Plate ID:

wavelength: 405

Temp:

Comments



Bio-Tek Instruments

Assay: Quick Read

Date: 03/07/03

Time: 12:57:28PM

Lot: AG 1-70 Plate 2

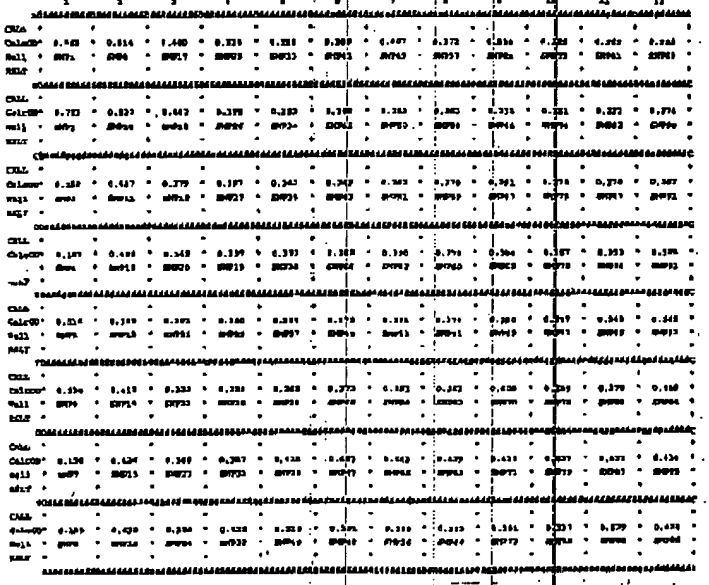
Operator:

Plate ID:

wavelength: 405

Temp:

COMMENTS



Continued on Page

Read and Understood By

Signed

3-2-03

Date

Signed

3-4-03

Date

PROJECT PGE2 Assay in AGS Cells

Notebook No. 2003-01

Continued From Page

Experiment 2003-01-23

Purpose + Procedure: The purpose is to look at the expression of PGE2 in AGS cells when exposed to multiple natural compounds. The procedure can be found on 2003-01-07

4.23.03 - The cells were plated at 7×10^5 cells per well in 2-96 well plates.

The following treatments will be used on the AGS cells. AGS cells - ATCC # HTB-137 lot 1641648

C1_COXAGS_04.21.03

AGS Cells - cells grown to confluence, wash, test materials added for 60 minutes then Az3187 [56 pM] added and assay for PGE2 35 minutes later.
For PGE2 assay run media diluted 1:20 only
4.21.03

Compound	Comments	d1 [ug/mL]	d2 [ug/mL]	d3 [ug/mL]	d4 [ug/mL]	No Wells
1. Isohop AN1127		50	5.0	0.5	0.05	8
2. 61115		50	5.0	0.5	0.05	8
3. Tetrahop AN1120		50	5.0	0.5	0.05	8
4. Hexahop AN1130		50	5.0	0.5	0.05	8
5. Alphahop AN1124		50	5.0	0.5	0.05	8
6. BetaHop AN1125		50	5.0	0.5	0.05	8
7. Iso-Rich AN1090		50	5.0	0.5	0.05	8
8. Tannin Extract #4411 AN1173		50	5.0	0.5	0.05	8
9. A70 LIPOTECH		50	5.0	0.5	0.05	8
10. Aromahop AN1126		50	5.0	0.5	0.05	8
					Total =	80

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Read and Understood By

Signed — Date: 4.23.03

Signed — Date: 4-26-03

PROJECT Antioxidant Study in HAEC cells Notebook No. 2003-01 45

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Experiment 2003-01-45

Purpose + Procedure 2003-01-30.

Antioxidant Testing in HAEC
Antioxidant assay using HAEC and 1000 µM H2O2+DMSO

Testing dates:		6/3/03	6/4/03	6/5/03		
No.	Test Material	d1 [µg/mL]	d2 [µg/mL]	d3 [µg/mL]	d4 [µg/mL]	No. Wells
1	Hopsteiner CO2 Hop Extract (AN1082)	100	10.0	1.00	0.10	8
2	Hopsteiner Beta AromaExtract Light Stable (AN1083)	100	10.0	1.00	0.10	8
3	5% Beta Hydrohop (AN1084)	100	10.0	1.00	0.10	8
4	5% Alpha Hydrohop (AN1085)	100	10.0	1.00	0.10	8
5	YC-Hop Aroma (AN1086)	100	10.0	1.00	0.10	8
6	YC-Alpharich (AN1087)	100	10.0	1.00	0.10	8
7	YC-TETRA (AN1088)	100	10.0	1.00	0.10	8
8	YC-Kettle RHO (AN1089)	100	10.0	1.00	0.10	8
9	ISO-Rich (AN1090)	100	10.0	1.00	0.10	8
10	YC-Purified Alpha	100	10.0	1.00	0.10	8
					total =	80

Plate 1

06.02.03/2_HAEC_ROI

10 · 100 10ul into 90ul

Antioxidant Testing in HAEC
Antioxidant assay using HAEC and 1000 µM H2O2+DMSO

Testing dates:		6/3/03	6/4/03	6/5/03		
No.	Test Material	d1 [µg/mL]	d2 [µg/mL]	d3 [µg/mL]	d4 [µg/mL]	No. Wells
1	BetaTech 1% Alphahop (AN1124)	100	10.0	1.00	0.10	8
2	BetaTech 1% BetaStab 10A (AN1125)	100	10.0	1.00	0.10	8
3	BetaTech 1% Aromahop (AN1126)	100	10.0	1.00	0.10	8
4	BetaTech 1% Isohop (AN1127)	100	10.0	1.00	0.10	8
5	BetaTech 1% Redhop (AN1128)	100	10.0	1.00	0.10	8
6	BetaTech 1% Tetrahop Gold (AN1129)	100	10.0	1.00	0.10	8
7	BetaTech 1% Hexahop Gold (AN1130)	100	10.0	1.00	0.10	8
8	BetaTech 1% Hops Oil (AN1131)	100	10.0	1.00	0.10	8
9	Mg Rho 60 (RtAA) (AN1176)	100	10.0	1.00	0.10	8
10	RtAA Hops #1199 (AN177)	100	10.0	1.00	0.10	8
					total =	80

Plate 2

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Read and Understood By

Signed 6-5-03 Date

Signed 6-6-03 Date

RAW

PROJECT 8.15.03 lysate on Cox-1, Cox-2, iNOS, IκB Plots Notebook No. 2003-04 22

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2003-04-22

Purpose: To look at Cox-1 Cox-2, iNOS, IκB in RAW cell lysates from 2003-03-41

Procedure:

4 Gels will be run using the Savant mini-electroblot system. Gels were removed from 4°C and rinsed in ultra-pure water. They were snapped into the Savant system and 1x SDS PAGE buffer was added. See 2003-04-08 for 1x SDS PAGE buffer.

4 sets of 0.5ml eppendorf self-locking tubes were set up. Equal volume of loading buffer Sigma S-3401 lot 21K5278 and protein sample (volume is in experiment 2003-03-41) were added to appropriate tubes. The tubes were shut and heated @ 100° C for 4 minutes to degrade proteins using a VWR standard Heatblock. The samples were then spun down briefly using an IEC micromax ultra-centrifuge.

The standard was added at 7ul to lane 1 of each gel (BioRad 161-0363 Precision plus Protein standard-Unstained) lot # 96182.

lane 2 - control
" 3 - LPS 10ng/ml
" 4 - RIAA 10ng/ml
" 5 - RIAA/LPS 10ug/10ng/ml
" 6 - Curcumin 10ug/ml
" 7 - Curcumin/RIAA 5ug/ml/5ug/ml TH LPS 10ng/10ng/ml
" 8 - Curcumin/RIAA 5ug/ml/5ug/ml
" 9 - Curcumin/RIAA/LPS 5ug/5ug/10ng/ml
" 11 - Caffeine 25ug/ml (used from experiment 2003-03-41)
" 12 - Caffeine/LPS 25ug/10ng/ml
" 10 - Caffeine/RIAA/LPS 5ug/5ug/10ng/ml
13 TH note order changed

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Read and Understood By

Signed 9-3-03 Date

9-5-03

PROJECT PGE2 Assay in RAW + HASC Notebook No. 2003-05 42

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2003-05-42

RAW PGE2 Procedure on Page 2003-05-03

C1_COXAGS_12.15.03

AGS Cells - cells grown to confluence, wash, test materials added for 60 minutes then A23187 [30 µM] added and assay for PGE2 30 minutes later.
For PGE2 assay run media diluted 1:20 only
12/15/03

Compound	Comments	d1 [µg/mL]	d2 [µg/mL]	d3 [µg/mL]	d4 [µg/mL]	No Wells
1. Salicin		50	5	0.5	0.05	8
2. New Kapvex		50	5	0.5	0.05	8
3. Old Kapvex		50	5	0.5	0.05	8
4. Pain Out		50	5	0.5	0.05	8
5. Joint Ease		50	5	0.5	0.05	8
6. Pharma Flam		50	5	0.5	0.05	8
7. Aspirin		50	5	0.5	0.05	8
8. APHS (Cayman)		50	5	0.5	0.05	8
					Total =	64

C2_COXAGS_12.15.03

AGS Cells - cells grown to confluence, wash, test materials added for 60 minutes then A23187 [30 µM] added and assay for PGE2 30 minutes later.
For PGE2 assay run media diluted 1:20 only
12.15.03

Compound	Comments	d1 [µg/mL]	d2 [µg/mL]	d3 [µg/mL]	d4 [µg/mL]	No Wells
1. Devils Claw 600 PLUS		50	5	0.5	0.05	8
2. LYMPHODRAN ORTHOPLEX		50	5	0.5	0.05	8
3. A.R.X. ORTHOPLEX		50	5.0	0.5	0.05	8
4. ARTHRO-COMPLEX		50	5.0	0.5	0.05	8
5. AR-ENCAP®		50	5.0	0.5	0.05	8
6. Traumagesic		50	5.0	0.5	0.05	8
7. Indomethacin		50	5.0	0.5	0.05	8
8. Celebrex®		50	5.0	0.5	0.05	8
					Total =	64

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Read and Understood By

Signed 12-15-03 Date

Signed 12-20-05 Date